# *Akkermansia muciniphila*

# A potential novel mechanism of nuciferine to improve hyperlipidemia

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ABSTRACT

*Background:* Intestinal microbiota is a novel drug target of metabolic diseases, especially for those with poor oral bioavailability. Nuciferine, with poor bioavailability, has an anti-hyperlipidemic effect at low dosages.

*Purpose:* In the present study, we aimed to explore the role of intestinal microbiota in the anti-hyperlipidemic function of nuciferine and identify the key bacterial targets that might confer the therapeutic actions.

*Methods:* The contribution of gut microbes in the anti-hyperlipidemic effect of nuciferine was evaluated by conventional and antibiotic-established pseudo-sterile mice. Whole-metagenome shotgun sequencing was used to characterize the changes in microbial communities by various agents.

*Results:* Nuciferine exhibited potent anti-hyperlipidemic and liver steatosis-alleviating effects at the doses of 7.5- 30 mg/kg. The beneficial effects of nuciferine were substantially abolished when combined with antibiotics. Metagenomic analysis showed that nuciferine significantly shifted the microbial structure, and the enrichment of *Akkermansia muciniphila* was closely related to the therapeutic effect of nuciferine.

*Conclusions:* Our results revealed that gut microbiota played an essential role in the anti-hyperlipidemic effect of nuciferine, and enrichment of *Akkermansia muciniphila* represented a key mechanism through which nuciferine exerted its therapeutic effects.

## 1. Introduction

Human life is dependent on a diverse community of intestinal microbiota that has co-evolved with their human hosts to modulate crucial aspects of digestion, immunity, nutrient acquisition, energy regulation, and maintenance of gut health. Growing evidence suggests that in the metabolic process, these symbionts regulate hormone sensitivity and gut barrier homeostasis on their own [1]. Simultaneously, the metabolites generated in bacterial metabolism can help us understand disease pathogenesis and reveal relevant pharmacological mechanisms against hyperlipidemia [2]. Accordingly, intestinal microbiota has great potential to become a novel target for the personal treatment of metabolic diseases.

Among various gut microbes, oral administration of *Akkermansia muciniphila*, a representative potential probiotic [3], improves the metabolic state and reduces the metabolic disorder induced by high-fat diet (HFD) [4]. To date, Akkermansia increases mucus thickness and intestinal barrier function, improves intestinal permeability, and reduces the inflammatory response caused by HFD, resulting in metabolism maintenance and immune response. Besides, the metabolic process can also lead to the production of beneficial short-chain fatty acids [5,6]. Therefore, Akkermansia has been accepted as a therapeutic target for lipid-regulating components due to its metabolic improvement [7,8]. Since then, the research on components that may promote Akkermansia growth through direct or indirect mechanisms has attracted great attention.

Nuciferine is a medicinal composition in lotus leaf (*Nelumbo nucifera*) and an effective isoquinoline alkaloid. It is effective in reducing blood and hepatic lipid [9] and ameliorating liver steatosis at low doses [10], and no obvious toxicities have been observed. Pharmaceutical studies have found molecular targets of nuciferine, such as PGC1α and PPARa [9–11], gradually elucidating the therapeutic mechanism of nuciferine in hyperlipidemia. However, nuciferine has a low bioavailability, which may affect the blood concentration, thus impairing its pharmacodynamics. A review of the pertinent literature has shown that drugs with poor bioavailability, such as curcumin, resveratrol [12], berberine [2], and diosgenin [13], exert

*Abbreviations:* Abs, antibiotics; AUC, area under the curve; CMC-Na, carboxymethylcellulose sodium; HFD, high-fat diet; LDL-c, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acid; NF, nuciferine; OGTT, oral glucose tolerance test; OTU, operation taxonomic units; PCoA, principal coordinate analysis; PCR, polymerase chain reaction; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα, peroxisome proliferator-activated receptor alpha; stat, simvastatin; TC, total cholesterol; TG, triglycerides.

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pharmacodynamic effects through altering the composition or metabolism of intestinal microbiota. Therefore, nuciferine, with poor bioavailability and similar structure to berberine, is also possible to exert a medicinal effect through modulating intestinal bacteria, such as Akkermansia spp. Here, we verified the antilipemic effect in the HFD-induced hyperlipidemia model, which was affected after antibiotic administration, suggesting that the effect partly depended on the intestinal flora. Moreover, we used metagenome technology and found the increased abundance of Akkermansia, indicating that Akkermansia might be the potential target of nuciferine. Collectively, our findings provided direct evidence that intestinal microbiota could become a target in the disease treatment.

## 2. Material and methods

### *2.1. Drugs and chemicals*

Nuciferine (purity≥98 %) was obtained from Kang Biotech Co., Ltd. (Hunan, China). Nuciferine was suspended in 0.5 % sodium carboxymethyl cellulose (CMC-Na). The HFD for rodents (H10060) with 60 % fat and heat energy was purchased from Beijing HFK Bioscience Co., Ltd. (Beijing, China). The normal rodent chow diet was obtained from Xietong Organism (Beijing, China). The compositions of the diets were listed in Table S1. Kits for total cholesterol (TC), triglyceride (TG), low- density lipoprotein cholesterol (LDL-c), and non-esterified fatty acid (NEFA) were supplied by Biosino Bio-Technology and Science Inc. (Beijing, China).

### *2.2. Animal experiments*

A total of 80 male C57BL/6 J mice (8 weeks old) were obtained from Beijing HFK Bioscience Co., Ltd. (Beijing, China). All animal-related protocols were approved by the research animal ethics committee of the Institute of Medicinal Plant Development affiliated to the Chinese Academy of Medical Sciences (Beijing, China) and performed according to the National Institutes of Health guide. Animals were placed in a semi- barrier system at room temperature and humidity with a 12− 12 h/light-dark photoperiod (from 7 a.m. to 7 p.m.). After 7 days of adaptative feeding, the mice were divided into eight groups (n = 10 in each group): Chow (normal chow diet), HFD (HFD diet), HFD + stat (HFD + simvastatin, 2.6 mg/kg, po), HFD + NF7.5 (HFD + nuciferine, 7.5 mg/kg, po), HFD + NF15 (HFD + nuciferine, 15 mg/kg, po), HFD + NF30 (HFD + nuciferine, 30 mg/kg, po), HFD + Abs (HFD + ampicillin + norfloxacin, 300 mg/kg per drug in drinking water), and HFD + Abs + NF15 (HFD + Abs + nuciferine, Abs in drinking water and nuciferine at 15 mg/kg, po). Animals were treated for 6 weeks. During this period, the mice were given free access to food and drink water, and the body weight was monitored once a week at a fixed time. Oral glucose tolerance test (OGTT) was carried out on day 36 after administration, and fecal samples were collected on day 38 and day 40. At the end of the experiment, the animals fasted overnight. The last gavage of tested drugs was performed at 8 a.m. on the last day, and then the blood samples are collected 1 h after administration. Animals were euthanized in chambers saturated with isoflurane and then sacrificed by cardiac puncture, and liver samples were harvested and stored at − 80 °C.

### *2.3. OGTT test*

After 35 days of the administration, OGTT tests were performed on overnight-fasted mice. Glucose (2 g/kg b.w.) was orally administered, and blood was obtained from the tail vein at 0 (before administration), 30, 60, and 120 min (after administration). The blood glucose levels were determined by a glucose meter (Roche, ACCU− CHEK Active, Branford, USA). Food supplements were resumed immediately after testing. The blood glucose change curve was drawn, and the area under the curve (AUC) was calculated by the area method under the trapezoid curve.

### *2.4. Measurement of serum parameters*

At the end of the experiment, the animals fasted overnight. After overnight fasting, the blood samples were collected at 1 h after drug administration, placed at room temperature, and centrifuged at 4500 rpm for 15 min at 4 °C. Serum was collected, and the levels of TC, TG, and LDL were determined using an automatic biochemical analyzer (Beckman Coulter, AU480).

### *2.5. Histological and pathological analyses*

The sections of the liver cut from the same position were immediately stored at -80℃ and observed by oil red O staining with cryotomy.

### *2.6. Estimation of lipid levels in the liver*

The sections of the liver cut from the same location were homogenized with anhydrous ethanol according to the instructions. After centrifugation, the supernatant was collected, and the relative lipid levels of TC, TG, and NEFA were determined according to the instructions of the kits.

### *2.7. Metagenomic sequencing*

Fecal samples were snap-frozen in liquid nitrogen and then stored at − 80 °C. After DNA extraction using a FastDNA® Spin Kit (MP Biomedicals, Santa Ana, US) and amplification of 16S rRNA V4–V5 regions by barcoded composition primers. Briefly, after an initial denaturation step at 95 °C for 5 min, the amplifications were carried out with 30 cycles at a melting temperature of 95 °C for 30 s, an annealing temperature of 55 °C for 30 s, and an extension temperature of 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The amplicons were pooled and purified using a QiaQuick PCR purification kit (Qiagen, Valencia, USA), and DNA pyrosequencing was sequenced using the Illumina sequencing platform. All samples were subjected to the bioinformatics data analysis process, including quality control, assembly, gene prediction and construction of non-redundant gene sets, gene function annotation, abundance quantification at the gene, species, and functional levels, flora composition analysis, diversity analysis, multi-

dimensional analysis, similarity analysis, and differential analysis.

### 2.8. Data analysis and statistics

For phylotype analysis, the alpha diversity of the microbiome was calculated based on the operational taxonomic unit (OTU) level by mothur (version 1.30.1). Principal coordinate analysis (PCoA) was performed using R and visualized by the R package (version 3.4.3, https: //www.r-project.org) to assess the similarities of metagenomic data among different groups. Their difference was evaluated by Adonis analysis, which is a permutational multivariate analysis that partitions the sums of squares using semi-metric distance matrices. Other significant differences were assessed using the unpaired Student's *t*-test. P- values were adjusted for multiple comparisons using the Benjamini- Hochberg false discovery rate and then further confirmed by post-hoc

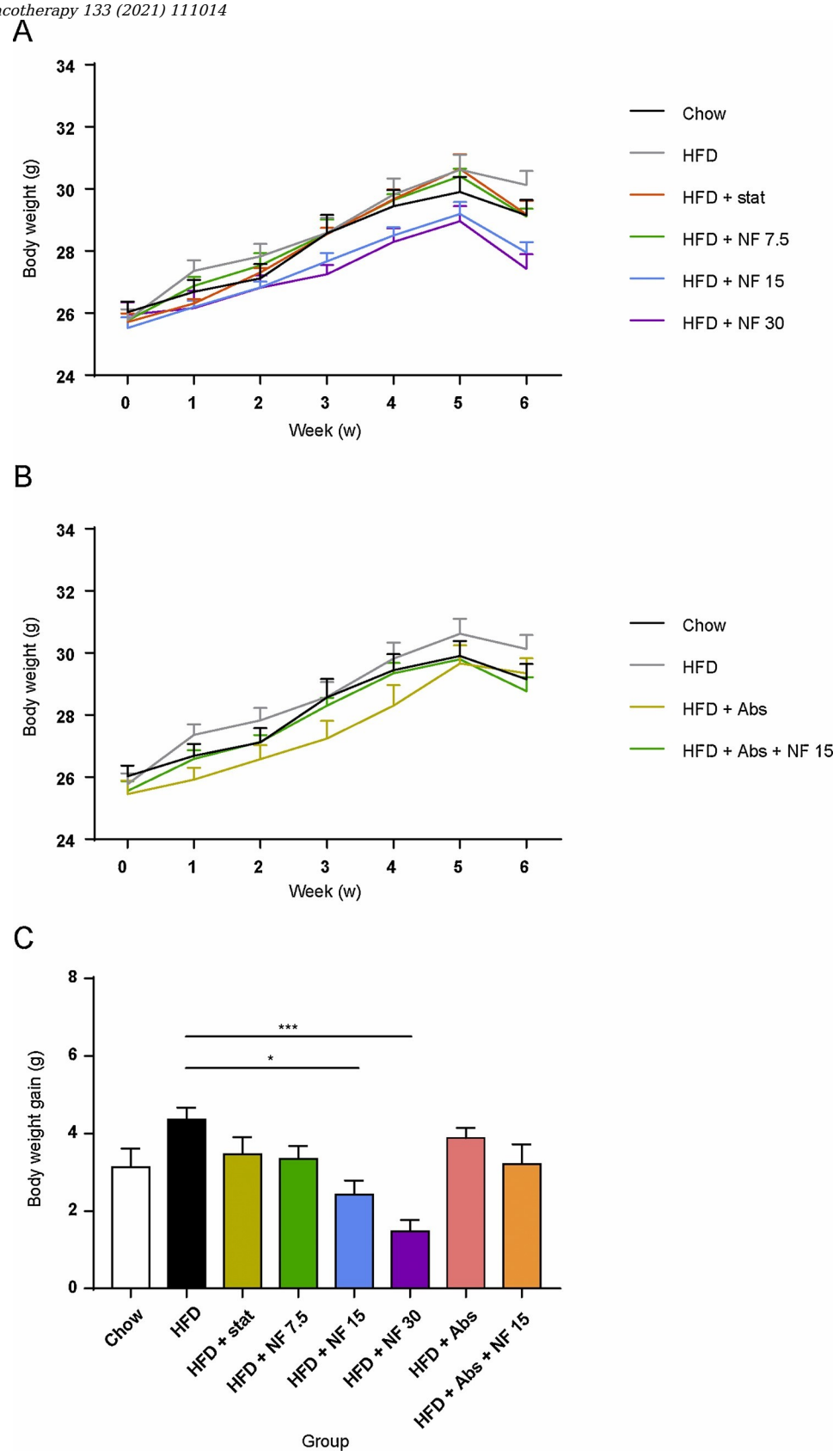


**Fig. 1.** Effect of nuciferine on HFD-induced body weight gain. (A) Body weight curve in mice without antibiotic treatment. (B) Body weight curve in mice with antibiotic treatment. (C) Body weight gain. (n = 9) Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns = no significant.

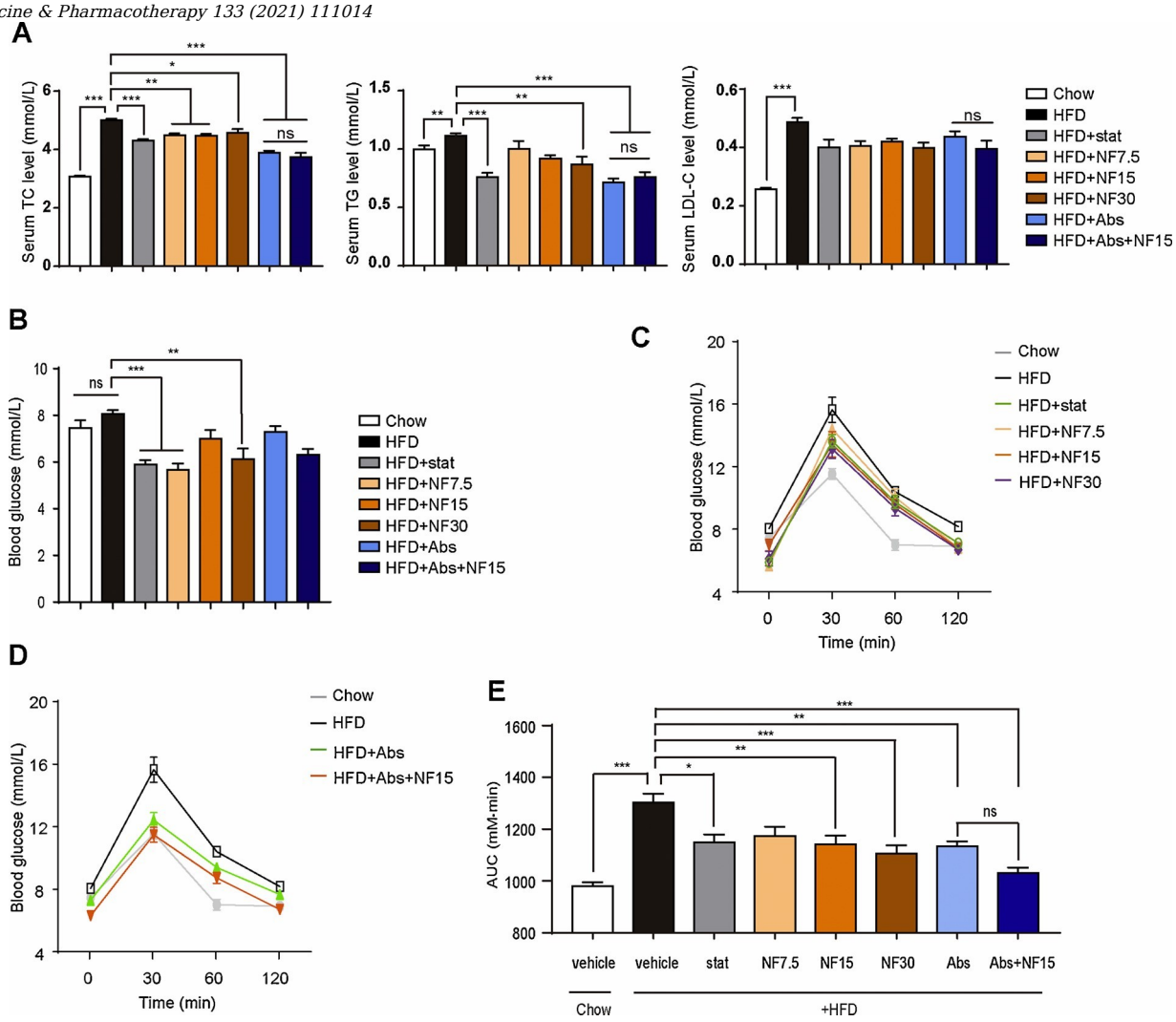


**Fig. 2.** Effect of nuciferine on serum lipid and glucose levels. (A) Serum lipid profile (n = 9). (B) Blood glucose levels at time point 0 of OGTT in each group (n = 8). (C) The OGTT curve in mice without antibiotic treatment (n = 8). (D) The OGTT curve in mice with antibiotic treatment (n = 8). (E) Glucose AUC (n = 8). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns = no significant.

analyses using Bonferroni correction. The significance was set at $P < 0.05$.

The data were presented as mean ± SEM. Lipid parameters statistical analysis was carried out by one-way ANOVA with Bonferroni's comparison (SPSS 25), and microbiome data analysis was carried out by *t*- test. A $P < 0.05$ was considered statistically significant.

## 3. Results

### *3.1. The anti-obesity effect of nuciferine is substantially deteriorated by antibiotics*

The body weight of each mouse was monitored during the experiment. HFD feeding markedly increased body weight as compared with the normal chow (Fig. 1A and 1C). Treatment with nuciferine significantly and dose-dependently inhibited the HFD-induced body weight gain, exhibiting more potent efficiency than the positive drug simvastatin (2.6 mg/kg) (Fig. 1A and 1C). Administration of antibiotics (ampicillin + norfloxacin, 300 mg/kg per drug) also reduced the body weight gain, while such reduction did not reach statistical significance (Fig. 1B and 1C). Co-treatment with nuciferine and antibiotics showed a minor decline in body weight gain compared with antibiotics alone. However, the inhibitory effect of nuciferine (15 mg/kg) on HFD-induced body weight gain was largely deteriorated (Fig. 1C). These results suggested that diminishing gut microbes by antibiotics substantially abolished the preventive effects of nuciferine on HFD-induced obesity.

### *3.2. Removal of gut microbiota abolishes the anti-hyperlipidemic but not the anti-hyperglycemic effect of nuciferine*

To verified the anti-hyperlidemic effect of nuciferine, the serum parameters were measured by an automatic biochemical analyzer. In this study, 6 weeks of HFD feeding resulted in remarkable hyperlipidemia in mice as manifested by dramatically increased serum levels of TC ($P < 0.001$), TG ($P < 0.01$), and LDL-c ($P < 0.001$) compared with the normal chow group (Fig. 2A). However, oral gavage of simvastatin and nuciferine significantly decreased serum lipid levels, exhibiting

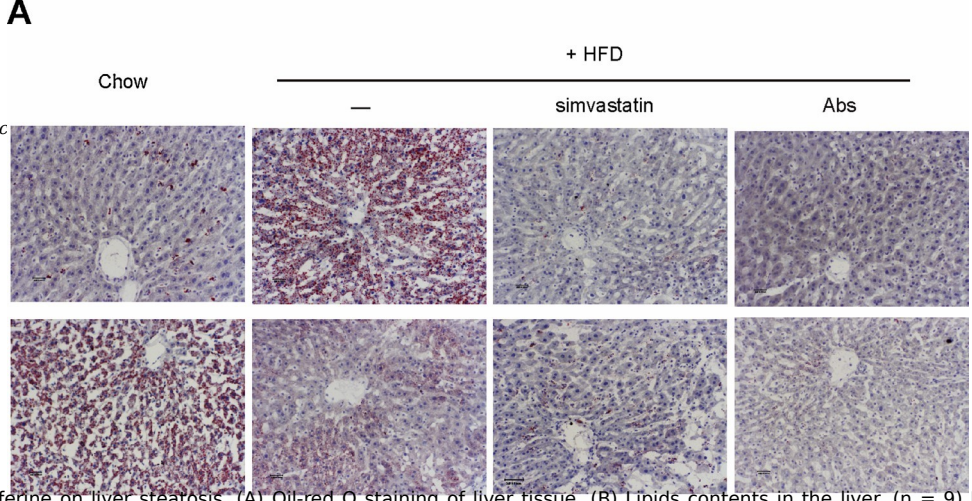


**Fig. 3.** Effect of nuciferine on liver steatosis. (A) Oil-red O staining of liver tissue. (B) Lipids contents in the liver. (n = 9) Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns = no significant (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

marked anti-hyperlipidemic effects (Fig. 2A). Next, we assessed the involvement of gut microbiota in the lipid-lowering effect of nuciferine via eliminating the gut microbes by-antibiotics and comparing the efficacy of nuciferine in both conventional and antibiotics-induced pseudo-sterile mice. Compared with the HFD group, removal of gut microbiota by antibiotic cocktail exhibited a more potent inhibitory effect on blood TC and TG ($P < 0.001$) levels, while it did not significantly decrease the blood LDL-c level (Fig. 2A). Notably, co-administration of antibiotics and nuciferine showed no additive effect compared with antibiotics alone, suggesting that nuciferine exerted a lipid-lowering action through a similar route with antibiotics. Moreover, the inhibitory effect of nuciferine on elevated serum LDL-c was substantially abolished after the clearance of gut microbiota. There was no synergy effect between nuciferine and antibiotics.

We further performed an OGTT to assess the impact of nuciferine on glucose metabolism and the potential involvement of the gut microbiota community. Even if feeding of HFD did not lead to hyperglycemia in mice (Fig. 2B), oral glucose tolerance was obviously impaired (Fig. 2 C–E). Nuciferine significantly improved oral glucose tolerance with a similar efficiency of simvastatin (Fig. 2 C–E). Besides, the antibiotics also largely improved oral glucose tolerance. However, in contrast to the above-mentioned findings, a combination of antibiotics and nuciferine showed a more pronounced improvement in oral glucose tolerance compared with antibiotics alone (Fig. 2 C–E), suggesting that nuciferine exerted an anti-hyperglycemic action through a gut microbiota-independent way.

### *3.3. The liver steatosis-alleviating effect of nuciferine is reduced by antibiotics*

To determine whether nuciferine had the same effect on liver steatosis, we analyzed oil red O signal, and liver lipid parameters generated from HFD feeding. HFD feeding led to significant liver steatosis as indicated by intensive oil red O signals (Fig. 3A) and increased liver contents of TC, TG, and NEFA compared with the normal chow group (Fig. 3B). The results showed that treatment with nuciferine largely ameliorated HFD-induced liver steatosis at the dose above 15 mg/kg (Fig. 3). Besides, we found that antibiotics also reduced the lipid accumulation in the liver, revealing an important role of gut microbes in the pathology of liver steatosis (Fig. 3A and 3B). Similar to the observation on serum lipids, a combination of nuciferine and antibiotics did not show a further therapeutic effect on the fatty liver compared with antibiotics alone. These results displayed that the beneficial effect of nuciferine on liver steatosis was, at least partially, attributed to the modulation of gut microbes.

### 3.4. Oral administration of nuciferine significantly shapes the overall structure of the gut microbiota

Bacteria are an integral component to maintain the stabilization of the gut environment. Therefore, to determine whether the effect of nuciferine on the host described above was also seen in the microbiota, we further checked the impact of nuciferine on the gut microbiota by shotgun metagenomic sequencing. Compared with the normal chow group, HFD, oral gavage of nuciferine or simvastatin did not significantly change the alpha diversity of the gut microbial community as revealed by Shannon and Simpson indices (Fig. 4A and 4B). However, antibiotics treatment significantly decreased the gut microbial variety compared with the HFD group as widely reported ($P < 0.001$, Fig. 4A and 4B). The PCoA illustrated that HFD dramatically shifted the overall structure of the gut microbiota. However, simvastatin exhibited no influence on the gut microbial structure, and nuciferine dose-dependently shifted the gut microbial community to the normal state (Fig. 4C). The modulatory effect of nuciferine on gut microbiota was essentially abolished by antibiotics, evidenced by that the data spots of the Abs + NF15 group were almost overlapped with those of the antibiotics group (Fig. 4C). These results indicated that nuciferine profoundly changed the gut microbial structure in HFD-fed mice.

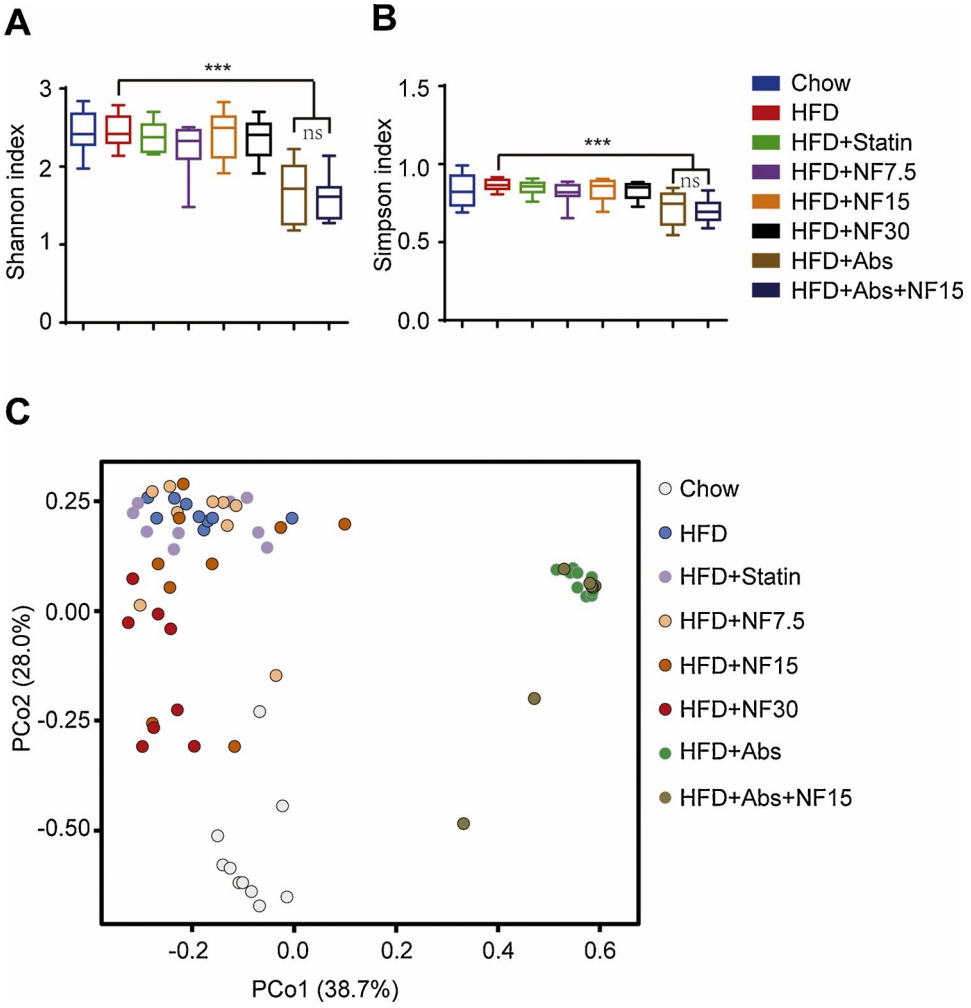


**Fig. 4.** Effect of nuciferine on the diversity and overall structure of the gut microbiota. The diversity of gut microbes was measured by Shannon (A) and Simpson (B) indexes. (C) The PCoA. (n = 9) Data are presented as mean ± SEM. ***P < 0.001, ns = no significant.

### 3.5. Enrichment of Akkermansia muciniphila and Bacteroides uniformis plays a key role in the anti-hyperlipidemic effect of nuciferine

Changes in microbiota composition among different groups were analyzed using individual taxon. HFD dramatically changed the composition of gut microbes with a marked decrease in beneficial genera compared with the normal chow group, such as Akkermansia and Bifidobacterium, and responses for an enormous increase in opportunistic genera, such as Helicobacter, Streptococcus, and Mucispirillum (Fig. 5A). Treatment with simvastatin showed little impacts on the composition of gut bacteria, which was consistent with its pharmacological mechanism (Fig. 5A), whereas oral administration of nuciferine markedly changed the gut microbiota, revealing that nuciferine profoundly shifted the structure of the gut microbiota. The most striking modulation by nuciferine was the restoration of Akkermansia and the decrease of Lactobacillus, while antibiotics greatly promoted the abundance of Bacteroides by 371 folds compared with the HFD group (Fig. 5A and 5B). We further inspected the

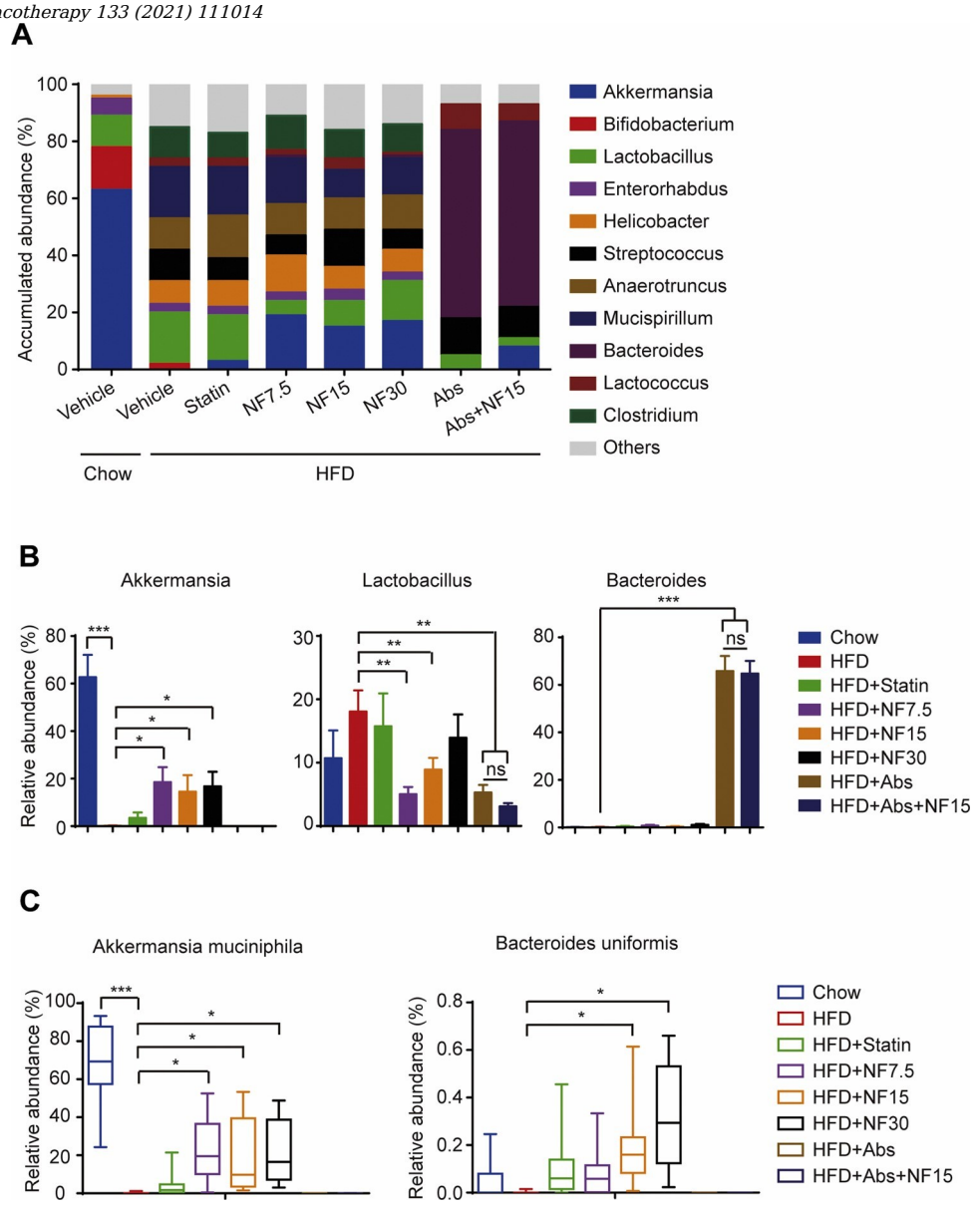


**Fig. 5.** Effect of nuciferine on the abundance of gut microbes. (A) Abundance profile of main genera in the gut microbial community. (B) The quantitative abundance of key genera that were significantly different among groups. (C) The specific gut bacterial species that were significantly modulated by nuciferine. (n = 8) Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns = no significant.

impact of nuciferine on gut bacteria at the species level (Fig. 5C). Downregulation of *Akkermansia muciniphila* and *Bacteroides uniformis* in the HFD group was significantly reversed after oral administration of nuciferine, and the abundance of Akkermansia was increased by 99 folds in the HFD + NF15 group ($P < 0.05$). These two species are well-documented to be effective to prevent HFD- induced hyperlipidemia [14,15]. The substantial increase of these two lipid-lowering species, especially *Akkermansia muciniphila*, by nuciferine suggested that they played a key role in the anti-hyperlipidemic effect of nuciferine.

## 4. Discussion

Although the notable lipid-reducing effect and a potential target of nuciferine have been discussed previously [9–11], it can become a novel research direction for microbiota due to its poor bioavailability. In our present study, the abundance of Akkermansia was increased after nuciferine administration in the hyperlipidemia model, while both the abundance and therapeutical effects could not be observed in pseudo-sterile mice, demonstrating that intestinal microbiota had the potential to be a novel target of anti-hyperlipidemic therapy.

As a systemic metabolic disorder, hyperlipidemia is characterized by dyslipidemia, which is mainly caused by genetic factor [16] and metabolic disorder [17]. To verify the anti-hyperlipidemic effect of nuciferine, firstly, an HFD-induced hyperlipidemia model was established, and different dosages of nuciferine all had an effect on regulating metabolic disorders. Also, the tendency obtained in lipid levels appeared in line
with the previous reports [9,10]. These data showed that for hyperlipidemia models, nuciferine had a comparable anti-hyperlipidemic effect as simvastatin, which could be used as a clinical drug [18].

To analyze the role of intestinal microbiota in nuciferine treatment, antibiotics were used to interfere with the gut microenvironment, and the Abs group and Abs + NF15 group were settled. Serum LDL-c and liver lipid parameters showed that antibiotic depletion of the microbiota eliminated the efficacy of the nuciferine, proving that the damage of intestinal microorganisms affected the efficacy of nuciferine. This finding confirmed that nuciferine acted on intestinal microbiota to have a lipid-lowering effect.

Metagenomic sequencing technology is a routine practice for scaling microbiome and metagenomic studies. To explore the reasons for the pharmacodynamic difference caused by antibiotics, the fecal samples were sequenced by this technique, and microbial diversity and PCoA were performed. Several researchers have reported that a diverse and stable microbiota may promote human health, while an imbalanced gut microenvironment will result in various peripheral diseases [19]. Even if the diversity was changed, HFD altered the structure and caused gut microbiota imbalance, evidenced by the decreased abundance of beneficial bacteria and the increased abundance of conditional pathogens. Based on the PCoA results, dose-dependent restoration of the microbial community in the nuciferine treatment groups couldn't be detected after antibiotic administration. Likewise, antibiotic treatment significantly reduced the diversity of intestinal microorganisms and altered their structure. Compared with the HFD group, oral administration of nuciferine significantly increased the abundances of *Akkermansia muciniphila* and *Bacteroides uniformis*. Aside from no synergistic effect between antibiotics and nuciferine, the abundance of Akkermansia was significantly reduced after antibiotic treatment, indicating that Akkermansia might be the key factor for the pharmacological effect. The subsequent experiments will be verified by enterobacterial transplantation technology.

These investigations, however, disregard the limitation of pseudo- sterile model. Commonly accepted oral antibiotics can change the biotransformation function of intestinal flora to drugs, leading to changes in drug efficacy [20]. Given that broad-spectrum antibiotics, a common method to cause a bacterial imbalance, may reduce the abundance and diversity of intestinal microorganisms [21], ampicillin and norfloxacin were selected to establish the pseudo-sterile model. Numerous studies have recognized that early intervention with antibiotics affects the metabolic system and increases the risk of obesity [22]. However, a lower lipid level and a better OGTT result were observed in our pseudo-sterile model, which was consistent with earlier studies in germ-free mice [21,23] and some detrimental effects observed in healthy mice and HFD-fed mice [24,25]. We speculated that an unexpected macrobiotic imbalance was made by killing the most beneficial and harmful intestinal bacteria at the same time, thus affecting the body's energy metabolism. Ideally, drugs are expected to kill or inhibit the growth of all harmful bacteria without affecting or impairing beneficial intestinal microorganisms. However, it is difficult to design targeted drugs due to the complex relationship between the flora and flora [26] as well as the interactions between the flora and host organism [27].

Taken together, our results revealed that Akkermansia was a potential target of nuciferine to ameliorate hyperlipidemia, providing novel insights into the lipid-lowering mechanism of nuciferine.

## Conclusions

Our results demonstrated the potential that intestinal microbiota might play an essential role in the anti-hyperlipidemic effect of nuciferine, and *Akkermansia muciniphila* was the potential key microbiome.

## Author contribution

Y.Y., X.L., XY.C., X.M., and M.H. carried out experiments and analyzed data. L.S. provided writing guidance. J. L., C.W., and X.C. designed research, provided guidance, and analyzed data. The histological and pathological analysis and metagenomic sequencing analysis were supported by Xuanwu Hospital Capital Medical University and QuantiHealth Co., Ltd., respectively.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.111014.

## References

[1] X. Tong, et al., Structural alteration of gut microbiota during the amelioration of human type 2 diabetes with hyperlipidemia by metformin and a traditional chinese herbal formula: a multicenter, randomized, Open Label Clinical Trial. mBio 9 (2018), https://doi.org/10.1128/mBio.02392-17.

[2] L. Zhu, et al., Berberine treatment increases Akkermansia in the gut and improves high-fat diet-induced atherosclerosis in Apoe(-/-) mice, Atherosclerosis 268 (2018) 117–126, https://doi.org/10.1016/j.atherosclerosis.2017.11.023.

[3] T. Zhang, Q. Li, L. Cheng, H. Buch, F. Zhang, Akkermansia muciniphila is a promising probiotic, Microb. Biotechnol. 12 (2019) 1109–1125, https://doi.org/ 10.1111/1751-7915.13410.

[4] A. Everard, et al., Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity, Proc. Natl. Acad. Sci. 110 (2013) 9066–9071.

[5] N. Ottman, S.Y. Geerlings, S. Aalvink, W.M. de Vos, C. Belzer, Action and function of Akkermansia muciniphila in microbiome ecology, health and disease, Best Pract. Res. Clin. Gastroenterol. 31 (2017) 637–642, https://doi.org/10.1016/j. bpg.2017.10.001.

[6] Y. Yang, et al., Early-life high-fat diet-induced obesity programs hippocampal development and cognitive functions via regulation of gut commensal Akkermansia muciniphila, Neuropsychopharmacology 44 (2019) 2054–2064, https://doi.org/10.1038/s41386-019-0437-1.
[7] J.J. Song, et al., Effects of microencapsulated Lactobacillus plantarum LIP-1 on the gut microbiota of hyperlipidaemic rats, Br. J. Nutr. 118 (2017) 481–492, https://doi.org/10.1017/S0007114517002380.
[8] K. He, et al., Rhizoma Coptidis alkaloids alleviate hyperlipidemia in B6 mice by modulating gut microbiota and bile acid pathways, Biochim Biophys Acta 1862 (2016), https://doi.org/10.1016/j.bbadis.2016.06.006, 1696-1709.
[9] F. Guo, et al., Nuciferine prevents hepatic steatosis and injury induced by a high-fat diet in hamsters, PLoS One 8 (2013), e63770, https://doi.org/10.1371/journal.pone.0063770.
[10] C. Zhang, et al., Nuciferine ameliorates hepatic steatosis in high-fat diet/ streptozocin-induced diabetic mice through a PPARalpha/PPARgamma coactivator-1alpha pathway, Br. J. Pharmacol. 175 (2018) 4218–4228, https://doi.org/10.1111/bph.14482.
[11] D.D. Zhang, et al., Nuciferine downregulates Per-Arnt-Sim kinase expression during its alleviation of lipogenesis and inflammation on oleic acid-induced hepatic steatosis in HepG2 cells, Front. Pharmacol. 6 (2015) 238, https://doi.org/10.3389/ fphar.2015.00238.
[12] Z. Gan, et al., Curcumin and resveratrol regulate intestinal Bacteria and alleviate intestinal inflammation in weaned piglets, Molecules 24 (2019), https://doi.org/10.3390/molecules24071220.
[13] M. Okawara, Y. Tokudome, H. Todo, K. Sugibayashi, F. Hashimoto, Effect of beta- cyclodextrin derivatives on the diosgenin absorption in Caco-2 cell monolayer and rats, Biol. Pharm. Bull. 37 (2014) 54–59, https://doi.org/10.1248/bpb.b13-00560. [14] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat. Rev. Microbiol. (2020), https://doi.org/10.1038/s41579-020-0433-9.
[15] S. Qiao, et al., Activation of a specific gut bacteroides-folate-Liver Axis benefits for the alleviation of nonalcoholic hepatic steatosis, Cell Rep. 32 (2020), 108005, https://doi.org/10.1016/j.celrep.2020.108005.
[16] A. Wiegman, et al., Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment, Eur. Heart J. 36 (2015) 2425–2437, https://doi.org/10.1093/eurheartj/ehv157.
[17] A. Pathil, J. Mueller, A. Warth, W. Chamulitrat, W. Stremmel, Ursodeoxycholyl lysophosphatidylethanolamide improves steatosis and inflammation in murine models of nonalcoholic fatty liver disease, Hepatology 55 (2012) 1369–1378, https://doi.org/10.1002/hep.25531.
[18] S. Karr, Epidemiology and management of hyperlipidemia, Am. J. Manag. Care 23 (2017) S139–S148.
[19] L.J. Spielman, D.L. Gibson, A. Klegeris, Unhealthy gut, unhealthy brain: the role of the intestinal microbiota in neurodegenerative diseases, Neurochem. Int. 120 (2018) 149–163, https://doi.org/10.1016/j.neuint.2018.08.005.
[20] D.H. Yoo, et al., Gut microbiota-mediated drug interactions between lovastatin and antibiotics, Drug Metab. Dispos. 42 (2014) 1508–1513, https://doi.org/10.1124/dmd.114.058354.
[21] B.S. Wostmann, C. Larkin, A. Moriarty, E. Bruckner-Kardoss, Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats, Lab. Anim. Sci. 33 (1983) 46–50.
[22] E. Isolauri, S. Rautava, S. Salminen, M.C. Collado, Early-life nutrition and microbiome development, Nestle Nutr. Inst. Workshop Ser. 90 (2019) 151–162, https://doi.org/10.1159/000490302.
[23] A. Zampa, et al., Effects of different digestible carbohydrates on bile acid metabolism and SCFA production by human gut micro-flora grown in an in vitro semi-continuous culture, Anaerobe 10 (2004) 19–26, https://doi.org/10.1016/j. anaerobe.2003.12.002.
[24] B.M. Carvalho, et al., Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice, Diabetologia 55 (2012) 2823–2834, https://doi.org/10.1007/s00125-012-2648-4.
[25] R.R. Rodrigues, et al., Antibiotic-induced alterations in gut microbiota are associated with changes in glucose metabolism in healthy mice, Front. Microbiol. 8 (2017) 2306, https://doi.org/10.3389/fmicb.2017.02306.
[26] M.A. Engevik, et al., Loss of NHE3 alters gut microbiota composition and influences Bacteroides thetaiotaomicron growth, Am. J. Physiol. Gastrointest. Liver Physiol. 305 (2013) G697–711, https://doi.org/10.1152/ajpgi.00184.2013.
[27] T.C. Fung, C.A. Olson, E.Y. Hsiao, Interactions between the microbiota, immune and nervous systems in health and disease, Nat. Neurosci. 20 (2017) 145–155, https://doi.org/10.1038/nn.4476.